United States Department of Agriculture
Bureau of Entomology and Plant Quarantine

A SUGGESTED METHOD FOR THE DETERMINATION OF THE ETHER-EXTRACTIVES CONTENT OF SMALL INSECTS

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Fulton[1] has published a microchemical method developed for the determination of the chloroform extractives of females of the beet leafhopper (Eutettix tenellus (Baker)) that is sufficiently sensitive to enable this content to be determined for as few as three individuals weighing about one-half milligram each. This method uses chloroform in a micro-Soxhlet extractor and requires the use of a micro-balance for the weighing.

Following suggestions from J. M. Fife, Biochemist at the laboratory of the Bureau of Plant Industry, U. S. Department of Agriculture, at Riverside, Calif., a simpler and more rapid method has been found that is sufficiently accurate for practical purposes, in which ether is used as the extractive agent.

The method described below requires a set of test tubes, small containers to hold the insects, an oven for drying the samples, and an analytical balance with a sensitivity of 0.1 mg for the weighing. In working with the beet leafhopper, which is a rather small insect, test tubes about 1 inch by 7 inches were used, and wooden racks were constructed capable of holding six tubes in a vertical position. The container for the insects is made of a piece of glass tubing about three-eighths inch inside diameter and 2 inches long. The lower end of this tube is covered with fine-mesh rayon voile, which is held in place by winding cotton thread around the voile and tube, as can be seen in figure 1. At the upper end of the tube a similar winding of cotton thread furnishes a point of attachment for a suspending thread, which is attached to a cork that fits the outer test tube tightly. In order to facilitate identification of the sample, a celluloid disk is cemented to the top of the cork with cellulose cement, and pertinent data can be written thereon with a glass-marking pencil.

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[1] Fulton, Robert A. 1937. Determination of chloroform extract of beet leafhopper. A micromethod. Indus. and Engin. Chem., Analyt. Ed. 9: 437-438, illus.

The procedure is as follows:

A sample of 15 or more female leafhoppers, weighing at least 4 mg, is air dried in the oven to a constant weight at 40° to 44° C. (104° to 113° F.). With leafhoppers this point is reached in about 5 days, but samples are ordinarily kept in the oven for a week to insure complete drying. The temperature of the oven should be kept below 50° C. (122° F.), as this temperature will melt some of the fat, as shown by a grease spot on the cardboard box containing the leafhoppers.

After being dried, the sample is cooled to room temperature and weighed on an ordinary analytical balance. The sensitivity of this type of balance is usually given as 0.1 mg, but the balance can be read to 0.01 mg if it is calibrated with known weights to determine the exact weight necessary to displace the pointer one dial space off center. The weighings are made by nearly balancing the sample with weights and then estimating the position of the midpoint from the average reading of several swings of the pointer in each direction. The displacement off center is multiplied by the value of one space as previously determined, and this reading added to or subtracted from the sum of the weights to secure the true weight. This is generally known as weighing by the "method of swings."

After being weighed, the whole insects, without any dissection or maceration, are placed in the small glass tube suspended inside the large test tube so that the lower end of the small tube dips into about 1 inch of ether previously placed in the bottom of the test tube. The test tube is then tightly corked, numbers are marked on the cork, and the test tube is placed in the wooden rack. After the insects have been left in the ether overnight, the small tube is withdrawn from the test tube and allowed to remain suspended from the cork in the open air for a few minutes to evaporate surplus ether. The insects are then replaced in their original cardboard containers and returned to the oven for 2 or 3 hours to drive off any remaining vestige of ether or moisture. A second weighing is then made, and the difference between the two weighings is the quantity of ether extractives.

The C. P. grade of ether "distilled over sodium" must be used. Ether boils at 34.5° C. (94.1° F.), a temperature often exceeded during the summer in many sections. For this reason it is highly desirable to conduct the extraction under cold conditions. Because of its low boiling point, ether is efficient as an extracting agent at temperatures close to or below freezing. A refrigerator should be used for the extractions if it is available. If the extractions are carried out under warm conditions, difficulty will be experienced with corks blowing out and ether evaporating. Ether is very explosive, so steps must be taken to avoid the accumulation of ether vapor, and adequate ventilation must be provided for the work of filling and emptying the test tubes.

Accuracy of the Method

Tests have shown that a single overnight extraction with the amount of ether indicated will remove practically all the extractives from a sample of 30 leafhoppers, containing from 5 to 10 mg. Unless extreme accuracy is required, a second extraction, with fresh ether, is not necessary. With larger insects, or with larger quantities of extractives present, it might be desirable to make a second extraction with fresh ether, using the discarded ether from the first extraction as the initial bath for a new sample of insects.

A large series of duplicate tests have been made, and the results of 58 pairs of tests, for which the average quantity of extractives per female was 0.109 mg, showed an average difference between duplicates of 0.009 $\pm$ 0.0001 mg, or about 8 percent.

For the extraction from females of the beet leafhopper it has been found that a sample of 15 females weighing from 6 to 8 mg is sufficiently large to give accurate results. The quantity of insect material needed would, of course, vary with the size of the individual insect and the amount of extractives present.

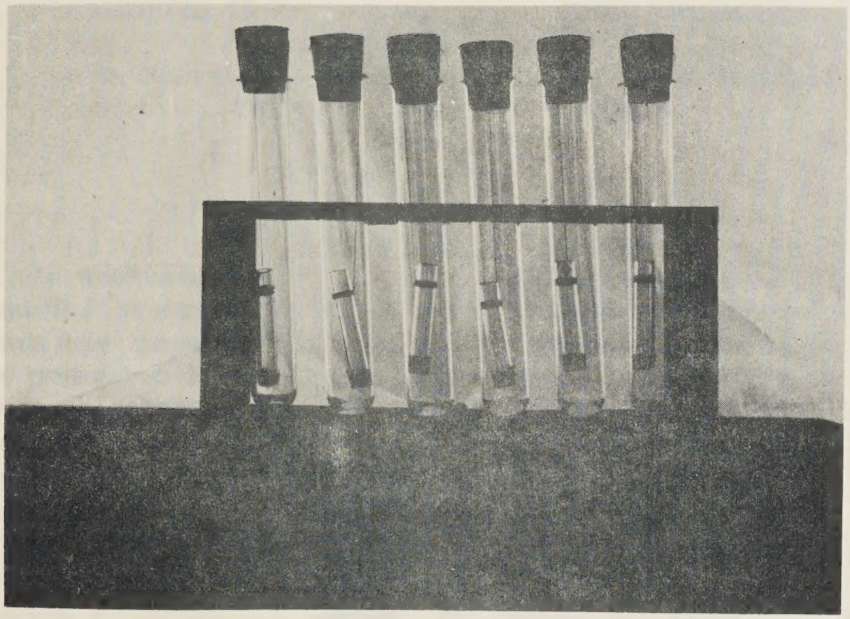

Figure 1.--Rack of test tubes set up for ether extraction. Small tube holding insects, open at top, closed at bottom with rayon voile, and suspended from the cork of the larger tube by a thread.